Project No. and Title: NE1707 Mycobacterial Diseases of Animals
Annual Meeting Dates: 02-December-2017

I. Participants

Last Name	First Name	Affiliation
Bannantine	John	NADC-USDA-ARS
Barletta	Raul	University of Nebraska–Lincoln
Bermudez	luiz	Oregon State
Chang	Yung Fu	Cornell University
Coussens	Paul	Michigan State University
Gerdts	Volker	VIDO-InterVac
Gibbons-Bergener	Suzanne	Wisconsin Department of Public Health
Grohn	Yrjo	Cornell University
Johnson	Peter	USDA-NIFA
Kapur	Vivek	Penn State
Katani	Robab	Penn State
Kerhli	Marcus	USDA-ARS/Iowa State University
Morrow	Alex	Centre for Agriculture and Biosciences International, UK
Olea-Popelka	Francisco	Colorado State University
Olson	Ken	AAMD
Patton	Elizabeth	Wisconsin Department of Ag
Quinn	Fred	Univ. of Georgia
Robbe-Austerman	Suelee	USDA-APHIS-NVSL
Smith	Rebecca	Cornell University
Sreevatsan	Srinand	Michigan State Univ.
Talaat	Adel	Univ. Wisconsin
Wagner	Bettina	Cornell University

The Fifth Annual Mycobacterial Diseases of Animals Multistate Initiative (MDA) was held in conjunction with the Conference of Research Workers in Animal Diseases (CRWAD) conference on Saturday, December 02, 2017 (7:00 AM to 6:00 PM) in the Sheffield Room at the Chicago Magnificent Mile, Chicago, IL. Besides registered participants, the conference drew close to a total of 40 interested researchers, students, and industry participants.

II. Welcoming Remarks:

Dr. Vivek Kapur (Chair; Penn State), Dr. Paul Coussens (Co-Chair; Michigan State), and Dr. Peter Johnson (National Program Leader; USDA-NIFA) each welcomed the participants, reviewed the objectives for the MDA establishment, and emphasized on the role of the annual MDA conference gatherings in highlighting progress and helping define key scientific issues relating to Mycobacterial diseases (primarily Johne's Diease and Tuberculosis) in animals. These diseases have significant impact on US animal biosecurity and food safety, and the conference helps highlight progress in various areas

including epidemiology, diagnostics, pathogen biology, immunology vaccine development, microbial genome diversity and evolution, pathogenomics and comparative genomics, functional genomics, host-pathogen interactions, approaches for disease prevention, and the development and delivery of educational and extension programming.

III. Plenary sessions:

The conference was organized as 12 plenary sessions.

Plenary session 1: An overview of Mycobacterium paratuberculosis
Presenter: John Bannantine (USDA)

Dr. Bannantine gave an overview of the past and present MAP related areas of research emphasis. He started his presentation by showing data indicating that there has been an increased in the JD related research and publications (~ 50%) due to the support from the USDA supported programs, including Johne's Disease Integerated Program (jdip) and its follow up entity, MDA. He then focused on the routes of MAP transmission, including colostrum, environment, within & between herds, and between species. Dr. Bannatine also reviewed some the new trends in the field of MAP. He presented the consensus statement from the Temple University (2017) on the role of paratuberculosis on the Crohn's Disease (CD). The attendees concluded that, 1) Map causes CD in genetically susceptible humans, 2) Koch's postulates have now been satisfied, and 3) FDA and USDA develop plans to rapidly eliminate Map from milk and meat supply. The two major control strategies are currently Testing/Culling, and Vaccination programs, but there are still other factors involved including farm management. Other areas of focus have also been on modeling MAP and diagnostics. For diagnostics, there have been several methods used to diagnose MAP, including antibody-based tests, nucleic acid based (IS_MAP04, IS900, IS_MAP02, and mbtA gene), and cell-mediated tests. Dr. Bannatine also reviewed the new and existing MAP-related challenges including, MAP association with MS patients, existence of live MAP in calf milk replacer (CMR), detection of MAP in powdered infant formula, and in bovine liver tissue (source of food for many countries), an all- voluntary control programs, and the existence of gap of knowledge and the low level of funding research for MAP research.

Plenary Session 2: An overview of Bovine TB and zoonotic TB.
Presenter: Fred Quinn (Univ. of Georgia)

Dr. Quinn's presentation focused on the Bovine Tuberculosis (bTB) and the potential correlates of Infection/Protection and relevance to human Tuberculosis. Dr. Quinn started by emphasizing on the impact of bTB as the pathogen (Mycobacterium bovis) infects > 50 Million cattle, costing > $3 billion / year. Typically, the infection correlate with the DTH response, but the it does not correlate with the severity of lesions, and the IFN-γ response does not correlate to the severity of lesions, he added. Dr. Quinn also added that studies have shown that in case of response to infection, IL-17A responses after challenge correlate with increased lesion severity, and in response to vaccination, IL-17A responses to PPD correlate with reduced lesion severity and IL-22 could be a predictor of vaccine efficacy. There have been many vaccination studies performed in the past 94 years, and so far, BCG is effective parenterally and orally, and with neonates. DIVA approach required and vaccines to prevent transmission to humans, livestock, and wildlife is needed. Moreover, Dr. Quinn evaluated the role of M. bovis and parasite co-infection and presented studies that have shown that animals with TB, if treated with anti-helminthic drugs were more likely to have macroscopic lesions

in the lungs, and thus, transmitted more effectively than controls, and anthelmintic treatment enhanced Th1 immunity, but the effect was insufficient to alter TB transmission risk. The studies have shown that the anthelmintic treatment could improve individual morbidity or mortality and simultaneously exacerbate TB transmission, and thus, enhance population-level spread of TB. At the end, Dr. Quinn focused on bTB and zoonotic TB, and emphasized that there is a need for multi-species diagnostics to prevent spread to multiple species.

Plenary Session 3: Accelerating control of bTB in developing countries.
Presenter: Vivek Kapur (Penn State University)

Dr. Kapur started the session with providing statistics on the current challenges of TB in India, as India has the largest burden of TB patients and deaths in the world (~500,000/year). Dr. Kapur also presented statistics on the bTB in India. bTB is endemic in India and there are no national control program or strategy. Dr. Kapur counted several key drivers of the emerging animal and public health crisis; including need for intensification of dairy production to meet increased demand and national priorities for nutritional improvement and rural development; test and cull approaches for bTB eradication not possible for India and most other developing countries; older age-structure of cattle in India from disincentives to cow slaughter; lack of approved vaccines or therapeutic approaches to prevent, cure, treat or reduce chances of spread of bTB infection in cattle; and consumption and handling of unprocessed and unpasteurized milk (> 70%) in India, primarily in rural and peri-urban areas. Dr. Kapur reviewed the currently emphasized efforts by the Melinda and Bill Gates Foundation to control bTB in the developing world. For instance, the foundation sponsored a workshop titled “Accelerating bTB Control in Developing Countries”, which was held on December 2015 in Rabat, Morocco. The strategies to control bTB as stablishing both the business case and the technical capabilities were the focus of the consortiums. This effort would lead to establishing sustainable market and policy drivers for implementation of national bTB control program. The group also focused on the needed tools, including better methods (vaccines) to prevent transmission and better Ante-mortem diagnostics. Dr. Kapur focused on the next steps to accomplish the goals. He is currently leading a project funded by the Gates’s foundation for bTB in both India and Ethiopia. The problem with current assays are that they are whole cell antigen based and difficult to manufacture (with low specificity) and cannot differentiate infected from vaccinated animals. The project focuses on the BCG efficacy and onward transmission trials and asks what is the efficacy of BCG vaccination and impact on onward transmission under natural exposure conditions?

Plenary Session 4 Develop and implement new generations of diagnostic tests.
4.1: for JD Bettina Wagner (Cornell University),
4.2: for TB Srinand Sreevatsan (Michigan State University)

4.1 Bettina Wagner’s topic of presentation was A new multiplex assay platform for serological differentiation of Johne’s disease stages. Dr. Wagner evaluated the challenges with the early diagnosis of MAP infection, including long sub-clinical phase, reliable diagnostic for infected animals during subclinical phase of Johne’s disease, intermittent shedding of MAP, slow growing in cultures, PCR detection of MAP in feces or milk current method of choice but poor sensitivity during subclinical stage, and late serological diagnosis of infected cattle with current ELISAs. Dr. Wagner introduced Multiplex testing. The advantages would be to have simultaneous detection, improved analytical sensitivity wider linear detection range, and advanced interpretation. Five recombinant MAP antigens, MAP1272c, MAP1569, MAP2121c, MAP2942c, MAP2609, and MAP1201c-2942c fusion protein were chosen based on the seroreactivity among candidate antigens within and

between the 4 groups (NL, NH, F+E-, F+E+), including some entirely novel candidate antigens (eg. Rv3696c_MAP0353 and Rv3695_MAP0356c) with considerable sero-reactivity during early infection and highly significant odds ratios. Comparison of individual MAP antigen-based assays indicated better differentiation of samples in the low positive range, improved analytical sensitivity, earlier detection of MAP infection especially with some antigens, and improved sensitivity and specificity even at individual assay level. Next steps would be to test additional antigens especially 'early antigens', perform multiplex assay optimization & selection of best MAP antigen combination, and multiplex assay validation including assay cut-off ranges and interpretation (AAVLD, A2LA, and OIE standards). Doing so, Dr. Wagner hopes to accomplish MAP antibody detection in milk and serum with improved sensitivity and specificity, early detection of MAP infection, advanced assay interpretation enabling proactive management of MAP infection, and tool for immune pathogenesis studies of Johne's disease.

4.2 Dr. Sreevatsan's topic of presentation was Addressing the challenge of tuberculosis in the animal-human interface - novel ideas in diagnostics. He focused initially on the evaluation of pathogen-specific biomarkers for the diagnosis of tuberculosis and the objective of his research is on developing a noninvasive biomarker-based detection system specific for *Mycobacterium bovis* for monitoring infection in animals and humans. He uses the pathogen peptide as a biomarker as it is a MTC-specific biomarker, can differentiate between different stages of the disease, and it has specificity and sensitivity in low/high disease prevalence areas. His research team use the *iTRAQ* - isobaric Tags for Relative Quantification to determine the amount of proteins from different sources in a single experiment. The pathogen proteins were selected based on their respective roles in pathogenesis and defense strategies and the positive and negative predictive values were plotted against percent prevalence, Dr. Sreevatsan explained. The results Different scales were used for different biomarkers, and some were highly elevated while others were not suggesting that the more elevated ones are better predictors. The overall results indicated that the experimentally infected deer show increasing biomarker trends, indicating the method works well as a biomarker for bTB monitoring.

Plenary Session 5: Increase understanding of the epidemiology and transmission of Mycobacterial diseases in animals, including predictive modeling.
Presenters: Yrjo Grohn (Cornell), Becky Smith (Univ. of Illinois)

The focus of this session was on the increase understanding of the epidemiology and transmission of Mycobacterial diseases including predictive modeling. The approach of this research is on developing a quantitative methodology for incorporating whole genome sequence data into bacterial transmission models for infectious diseases incorporating ecology, economics, molecular biology, and epidemiology, and to apply these methods and models towards better understanding the principles and dynamics governing transmission of mycobacterial infection. The transmission modeling was conducted using the A multi-group compartmental SIR (susceptible, infectious, resistant) model. Dr. Grohn explained that the current MAP control options are "Test and remove", "Hygiene", "Nothing (cull on production)". Studies have shown that Testing and Culling do not eliminate MAP from herd. Along with collaborators from other universities and USDA, Dr. Grohn and Dr. Smith have worked on a joint project modeling bTB, within herd and between herd. One objective of the project is to use Whole Genome Sequencing of infected animals and epidemiological data to jointly estimate *M. bovis* transmission in MN cattle herds and deer population. The results indicated that there are four major clades that could not be distinguished by sampling time, host-species, nor sample location. The results also indicated that elk in Michigan not

a significant source of bTB transmission, and WGS provided the resolution to genetically distinguish *M. bovis* isolates and with this study we will be better informed about the necessity of establishing new bTB management programs in this area. A second study that Dr. Grohn's research group work focus on is the understanding historic and contemporary bTB transmission dynamics in Michigan. The analysis from the study showed that *M. bovis* genetically distinct groups could not be identified by the species they were sampled from, suggesting that this admixture could be due to cross-species transmission, providing evidence of transmission between deer and cattle and deer and elk.

Plenary Session 6: Pathogenesis of bTB and Johne's.
Presenters:
6.1 Raul Barletta (University of Nebraska–Lincoln), "Molecular Genomics and Pathogenesis of Mycobacterium avium subspecies paratuberculosis".

Dr. Barletta's research focuses on the MAP K-10 MycomarT7 mutant library generation. A transposon insertion "hot spot" downstream from the MAP_4116c (mmaA4) gene resulted in a biased identification of essential genes. Data re-normalization by a geometric means beta distribution eliminated the "hot spot" anomaly, identifying 111 essential genes by HMM analysis. Establishment of a "low dose" MAP infection model detected no humoral immunity but the IFN-g test indicated that both K-10 and the mutant pool animals were positive for MAP infection. Passive fecal shedding was seen during the first 30 days post-infection. Intestinal tissues, and their associated lymph nodes, liver and spleen were colonized with MAP up to approximately 4,000 CFU/g of tissue on average for the mutant pool and 1,000 CFU/g of tissue on average for K-10. Mutant pool infection displayed similar characteristics to the wild type infection - most transposon mutants have a wild type infection phenotype as expected. Dr. Barletta concluded his talk with a focus on the future studies in developing a live attenuated (two attenuating mutations) unmarked (no antibiotic resistant markers) DIVA (differentiating infected from vaccinated animals) vaccine against JD in cattle that avoids *M. bovis* cross-reactivity and establishing JD diagnostic tests for cellular and humoral immunity in conjunction with the DIVA formulation.

6.2 Luiz Bermudez (Oregon State University), "The challenges of studying and addressing *Mycobacterium bovis* disease". Dr. Bermudez pointed out that animals get infected primarily through the respiratory and digestive tracts, and treatment with the current therapeutic arsenal is impractical, and prevention of infection by improving the innate response is a promising strategy. Dr. Bermudez's research group focused on the *M.bovis* interaction with mucosal cells, the bacterial surface expression, and the adaptive (Ig)Immune Response. Dr. Bermudez also presented some freeze-fracture Transmission electron microscopy of *M.bovis* infected bovine macrophages.

Plenary Session 7: Zoonotic risks associated with of Mycobacterial diseases in animals.
Presenters: Francisco Olea-Popelka (Colorado State University), "Zoonotic risks associated with of Mycobacterial diseases in animals".
Dr. Olea-Popelka reviewed the risk of ZTB increases for people living in communities with higher prevalence of bovine TB in livestock, lack of milk pasteurization, and human immunodeficiency virus (HIV). He further pointed out that the people at risk and affected by ZTB are the most neglected, living in rural areas far away from health centers. Statistics from Emerging Infectious Diseases show that "During 2010–2011, case-patients with *M. bovis* disease were more likely than those with M. tuberculosis disease to die before treatment completion (15.8% vs. 8.6%, p = 0.006)". Dr. reviewed a summary of why it is important to distinguish *M. bovis* as an agent of human tuberculosis. The list includes the uncertainty of the true incidence of ZTB, the clinical features of

ZTB present being challenges for patient treatment and recovery, the fact that *M. bovis* is naturally resistant to pyrazinamide, and can acquire resistance to other TB drugs, and *M. bovis* infection in humans is mostly foodborne, and ZTB in humans is associated with extra-pulmonary TB.

Plenary Session 8: Public health risks associated with *M. bovis* in Mexico (foodborne and occupational health risks).
Presenter: SueLee Robbe-Austerman (USDA)

Dr. Robbe-Austerman evaluated the genetic relatedness of bTB outbreaks in the USA in the last 20 years and presented few cases of collaborative effort between USDA and international collaborators. For instance, a case study was evaluated between Mexico and USA and the project involved whole genome sequencing(WGS) and single nucleotide polymorphisms (SNP) analysis, followed by phylogenetics of bTB in North America and Baja CA, Mexico. The study concluded that 95% of human cases in Baja CA and Southern CA originate from cows in the Baja CA non-accredited zone. Additionally, the study suggested that there are unique events that cause most of the spill over into humans, and little evidence of human to human transmission was determined. The data also suggested that possible human to cow transmission may have occurred.

Plenary Session 9: Develop programs to evaluate and develop new generations of vaccines for JD and TBc. “Protecting Canada and the world from infectious diseases”.
Presenter: Volker Gerdts (VIDO-InterVac)

Dr. Gerdts introduced the concept of Reverse Vaccinology Approach for the Prevention of Mycobacterial Disease in Cattle (ReVAMP). The goals of the project are to subunit vaccine (cost-effective, easy to produce, stable, depends on adjuvants), find DIVA potential (trade issues), and companion diagnostics (ELISA-based). The objectives for ReVAMP are to use reverse vaccinology, diagnostics, host response to MAP infection, and GeLS^3 (socioeconomic). The study was conducted both for JD and bTB starting with genome, bioinformatics, component identification, potential antigen cloning, testing in gut loop models, and vaccine testing development and commercialization. The study is to focus on DIVA vaccine and antigens that are highly immunogenic, unique, induce both humoral and cell-mediated responses, and does not interfere with Skin-test

Plenary Session 10: Novel vaccines for JD.
Presenter: Adel Talaat, University of Wisconsin-Madison

Dr. Talaat’s research group focuses on the attenuated pathogens missing virulence or metabolism genes, by design or through passaging, maintain a balance between attenuation and virulence (M. tb vs. BCG), vaccine strain selection, and rational vaccine design, based on mechanistic studies. Dr. Talaat emphasized the benefits of using a live Attenuated Vaccine (LAV) as follows; easy to produce & inexpensive, longer persistence, no adjuvant, and long-term, comprehensive immunity. The cons in using LAV were counted as safety (reversion, gain of virulence, immunocompromised patients), efficacy: strain Bkg, target population. Dr. Talaat concluded that for LAV, there is a need to maintain a balance (attenuation vs. immunogenicity), use novel candidates with M. tb Bkg (e.g MTBVAC, ΔmosR, ΔechA7) improved over BCG, novel LAV against *M. bovis*, and improve working with modelers for immune responses and disease transmission.

Plenary Session 11: An introduction to the Global Research Alliance for Bovine Tuberculosis (GRAbTB) network.

Presenter: Alex Morrow (Centre for Agriculture and Biosciences International, UK)

Dr. Morrow initiated his talk by reviewing GRAbTB, a global initiative to address the coordination of research programs at international level in the area of animal health and in particular infectious animal diseases including zoonoses. The program is a four-year EU-funded global initiative to address the coordination of research programs at international level STAR-IDAZ had 25 partners from 18 countries. Regional networks were established for a) the Americas, b) Asia and Australasia, and d) Africa and the Middle East complementing the existing European network – so a total outreach of = > 50 countries. Network now operating under an MoU signed, as of 27/4/2016, by 28 funding organizations from 21 countries. bTB workshops have been established in different areas, including vaccines, diagnostics, host-pathogen interactions, and epidemiology. The GRAbTB has a vision of coordinated global research alliance enabling improved understanding and control of bovine TB, and a mission to establish and sustain global research partnerships that will generate scientific knowledge and tools to contribute to the successful control and eradication of bovine TB. It also has four goals to identify research opportunities and facilitate collaborations within the Alliance, conduct strategic and multi-disciplinary research to better understand bovine TB, develop novel and improved tools to control bovine TB, serve as a communication and technology sharing gateway for the global bovine TB research community and stakeholders, and promote collaboration with the human TB research community.

Plenary Session 12: Extension/Outreach/Human Disease: “One Health: Wisconsin’s Collaborative Response to Tuberculosis”.

12.1 Presenters: Elisabeth Patton (Wisconsin Dept. Ag. Trade and Consumer Protection), and Dr. Suzanne Gibbons-Bergener, WI Department of Public Health.

Dr. Patton and Dr. Gibbons-Bergener examined the TB response role of Wisconsin Department of Agriculture. Response planning involved collaborators from the division of Public Health, division of Animal Health, and Department of Natural Resources. TB testing in cattle involves two methods; caudal fold tuberculin test (CFT), which involves screening test for bovine TB, and purified protein derivative of bovine tuberculin injected intradermally under the tail base. Another method is comparative cervical tuberculin test (CCT), which involves confirmatory test for bovine TB, and purified protein derivatives of bovine and avian tuberculin injected intradermally in the neck. Other activities involve a joint DAH/DPH brochure about bovine TB was produced in 2016 to increase livestock owner awareness about the human and animal risks associated with the disease, as well as the resources available through the Wisconsin TB Program. There are also challenges involved, including coordinating response with local health departments and field veterinary staff, lack of access to health care for farm workers, communicating with farm workers and owners, identifying funding sources for response and case management, and compliance with health record privacy laws. Dr. Patton reviewed the future directions as to refine our notification, assessment, and testing protocols to including, communication/action checklist, animal worker questionnaire to identify elevated-risk tasks, animal risk assessment tool, inclusion of occupational health programs for abattoir workers, expanded interagency zoonotic disease communication plan, and targeted TB screening and testing programs for animal workers.

12.2 Develop and deliver education and outreach material related to JD and TBc in electronic and print form for use by various stakeholders. Ken Olson (KEO Consulting)

Dr. Olson provided some information on the Johne's Information as follows: NVJDCP provided incentive for materials and JDIP was a major contributor, with loss of funding interest has lagged, but programs operate in several states, and it is still an area of concern for producers in the US and around the world. Dr. Olson emphasized that both Johne's and TB are reportable, but there is an national eradication program for TB and a voluntary program for Johne's. MDA has been involved in several other areas including, DHIA – QCS, NYSCHAP Johne's Disease Module, Johne's Information Center UW - School of Vet Medicine, USDA- Johne's Disease Information, Michigan Department of Natural Resources, Indiana BOAH, and Center for Food Security & Public Health – ISU.

Closing Remarks:

Closing remarks, planning the next steps, and wrap up were presented by Paul Coussens, Ken Olson, Marcus Kehrli(USDA-ARS-NADC), and Peter Johnson (USDA-NIFA).

The meeting was adjourned at 6:00 pm.